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RNA-seq based expression analysis of the CHO cell protein secretion pathway

Anne Mathilde Lund^{1, *}, Christian Schröder Kaas², Helene Fastrup Kildegaard³, Claus Kristensen², Mikael Rørdam Andersen¹

(1) Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; (2) Current address: Novo Nordisk, Maaloev, Denmark; (3) Current address: Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hoersholm, Denmark
(*) amalu@bio.dtu.dk

Introduction

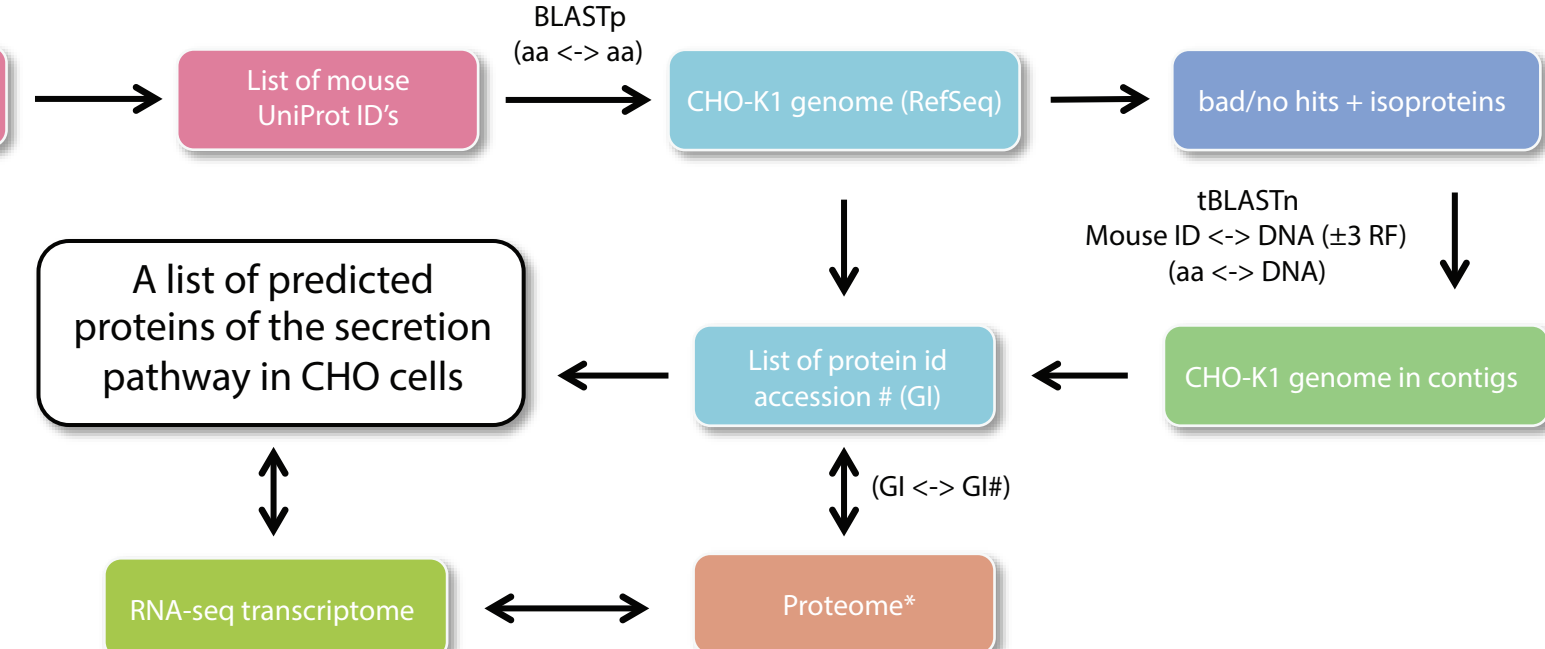
The Chinese hamster ovary (CHO) cell-line is the predominant mammalian industrial cell line being used to produce recombinant therapeutic proteins. Although CHO cells have been used for more than 25 years, the genome sequence was first published in 2011. So far there have been limited studies of the cell biology of the CHO cell and the potential of cell line engineering. To elucidate the poorly understood cellular processes that control and limit recombinant protein production and secretion, a system-wide study was initiated to identify possible engineering targets relevant for therapeutic protein production.

Objectives

- Reconstruction of the complex cellular machineries of the early protein secretion pathway and unfolded protein responses by employing legacy knowledge of mouse
- By using RNA-seq data, a differential gene expression analysis of the constructed CHO secretion pathway would provide a unique possibility for identification of active components to increase the productivity of recombinant proteins.

Strategy of *in silico* reconstruction of CHO cell pathways

First a study was initiated for identifying mouse proteins in the literature associated with the secretion pathway and the ER stress responses. Amino acid sequences were used for BLASTp against the available RefSeq CHO-K1 genome. The identified genes could be used for extracting parts of the mapped RNA-seq data. However, only mapped genes



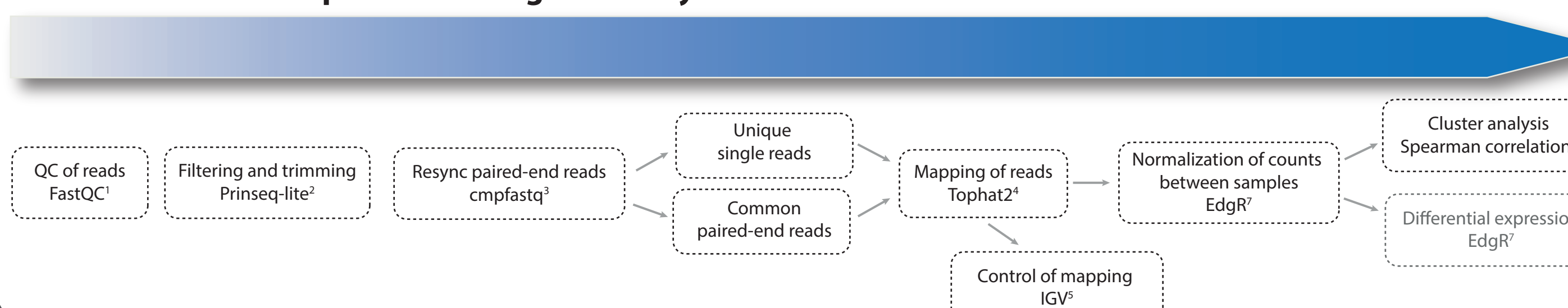
Design and methods for RNA-Seq samples

- RNA was extracted under different growth conditions and treatment from three different CHO cell lines
- Paired-end RNA sequencing was performed by AROS a/s on Illumina HiSeq 2000 platform with a sequencing depth of min. 35 mio reads

Overview of samples

CHO-K1_none		CHO-K1_IgG				CHO-DG44_FVIII		
exp. growth	stationary	exp. growth	stationary	NaBu		exponential growth		
1 samples	1 samples	control	0%NEAA	control	0%NEAA	2 samples	none	medium
		1 sample	1 sample	1 sample	1 sample		5 samples	11 samples
								2 samples

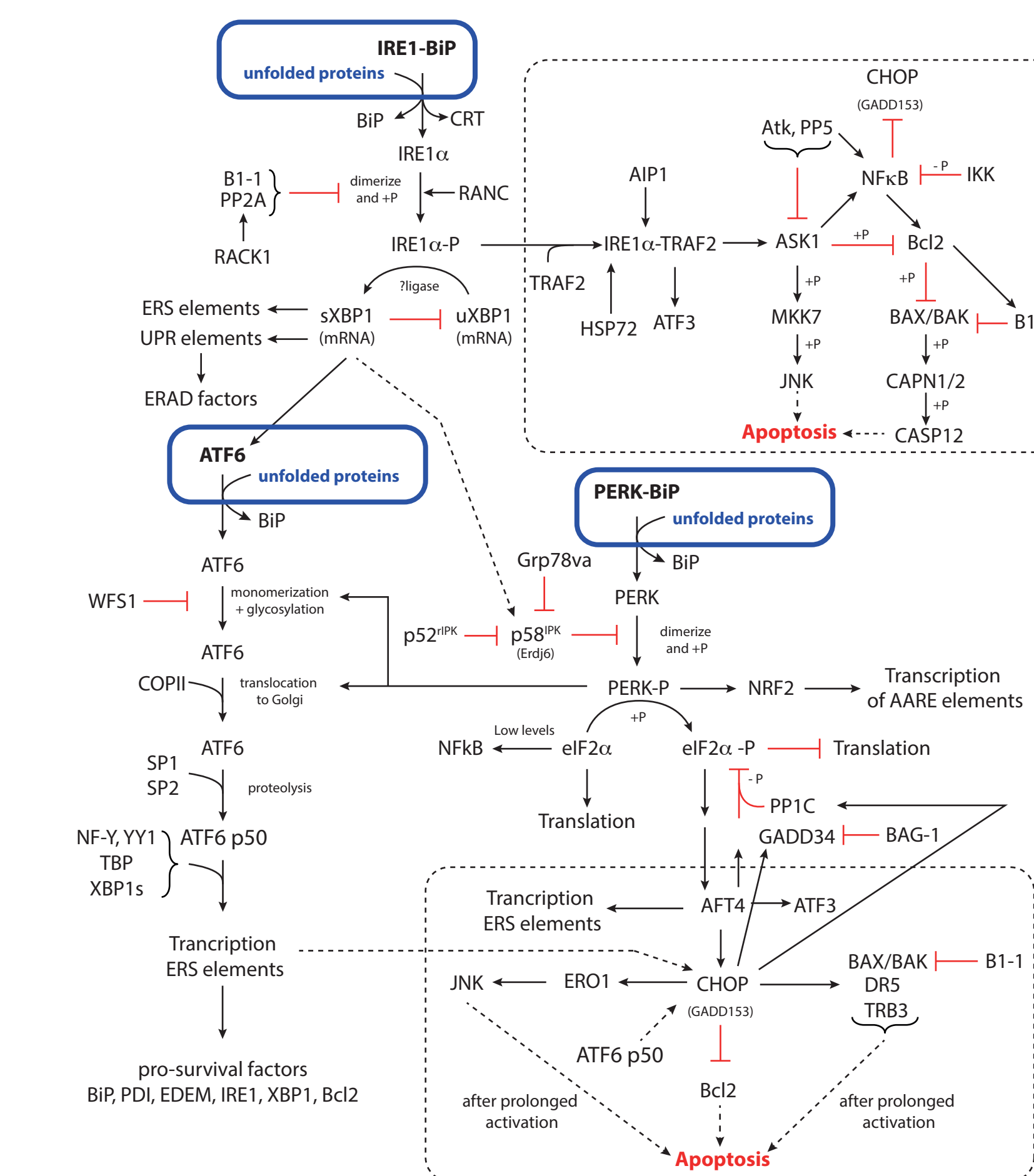
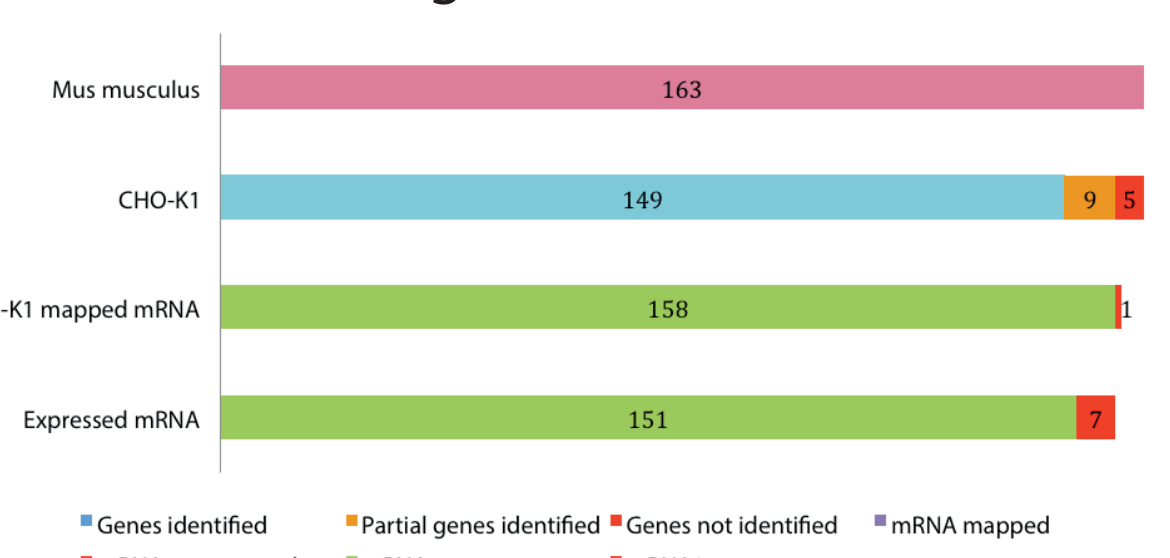
Workflow of RNA-Seq data handling and analysis



Reconstructed UPR pathway and RNA-Seq data analysis

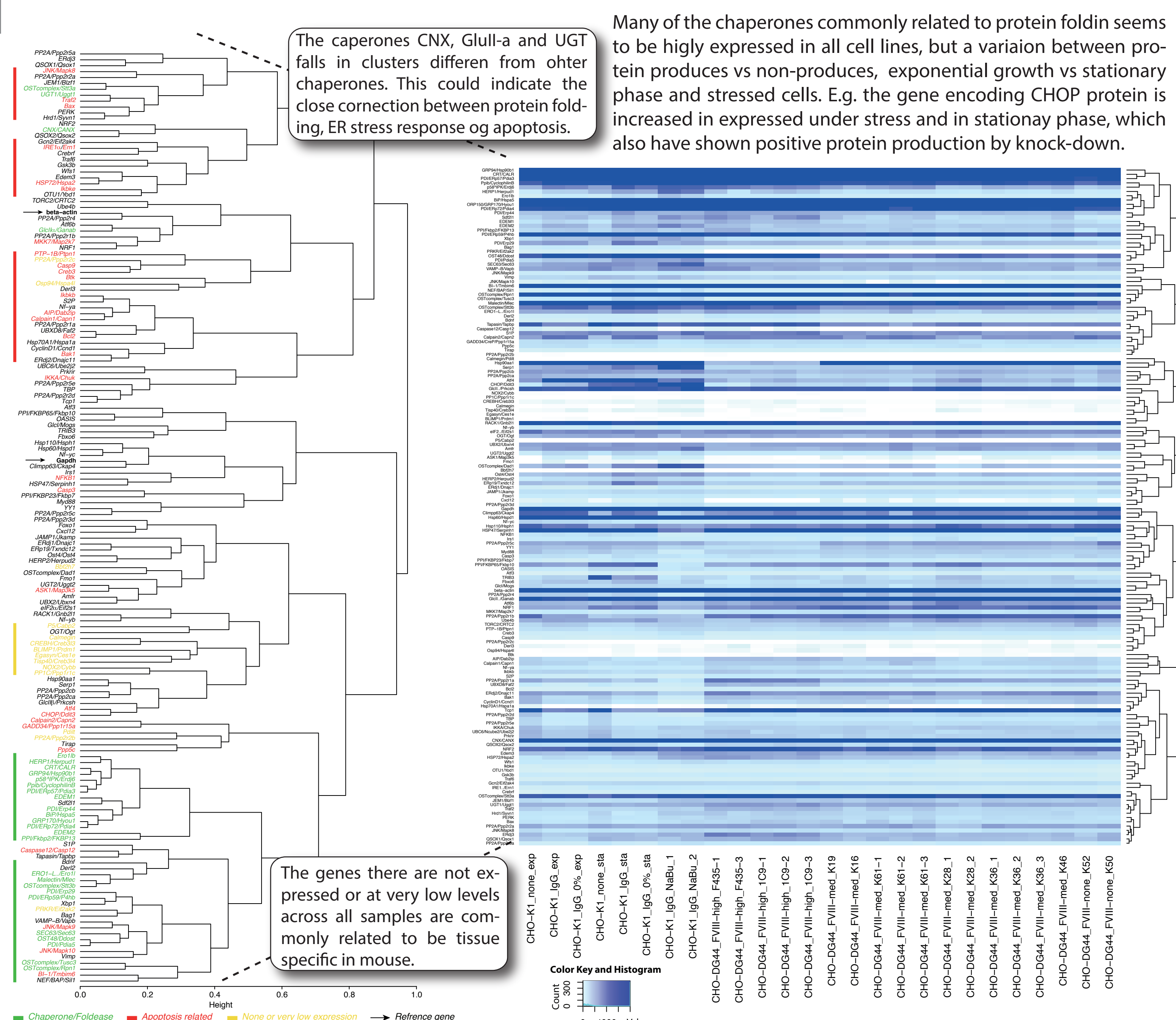
Proteins associated or linked to UPR pathway were identified by manually curate available literature on mouse models and cells lines. Furthermore, was the know interactions and dynamics of proteins in the UPR pathway mapped. Found proteins were used to identify CHO-K1 genes of the UPR pathway.

Identified genes and mRNA of CHO-K1



Protein known to be involved in the apoptotic pathway and have a direct interaction is also seen to cluster across the samples in the RNA-Seq data. However, apoptosis can be induced through different path of the UPR pathway dependent on the cause of stress, which is likely the reason for some of the apoptosis genes to clustered more closely to chaperones and folding proteins

The CHO-K1 genome was sequenced recently and the annotation is at present insufficient, so more than 4 mio. reads was from each sample not possible to map.



Perspectives

This preliminary study shows the possibilities for using RNA-seq data and cluster analysis to identify new gene clusters based on biological gene expression behaviour that can lead to a greater biological understanding of the important industrial cell CHO-K1. It can be used for identifying genetic targets for improvement of protein production by overexpression transcription factors or create knock-downs of growth inhibiting.

References

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